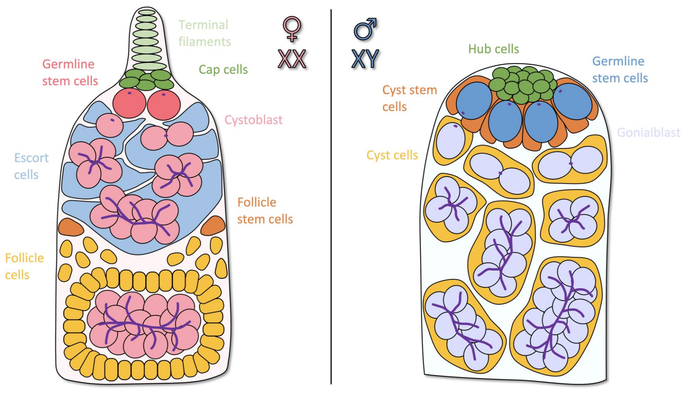
Characterizing the Role of Doublesex in Creating Sexual Dimorphism in the *Drosophila* Gonad

Natalie A. Murphy, Ellen Baxter & Mark Van Doren

Department of Biology, Johns Hopkins University, Baltimore, Maryland, USA

Introduction

Across the animal kingdom, sex determination is controlled by the Doublesex and Mab-3 Related Transcription factor (DMRT) family proteins. In *Drosophila*, sex determination is regulated by X chromosome dosage, which activates an alternative splicing cascade to produce Dsx^F in females and the default Dsx^M in males. Both Dsx isoforms have the same DNA binding domain, but they regulate their targets differently to yield sexual dimorphism [Camara et al., 2008].



The embryonic gonad initially forms as a bipotent cluster of somatic gonadal precursors (SGPs) that has coalesced with the germ cells. It is known that *dsx* is expressed in the SGPs during embryonic gonad development, where it acts to control their sex-specific development. In the male, important somatic cell types include the stem cell niche (the hub) and the cyst stem cells, while in females they include the stem cell niche (the terminal filaments and cap cells), the escort cells, and the follicle stem cells. We are curious as to how Dsx regulates SGP development to produce these key cell types of the ovary versus the testis.

Where is Dsx Expressed in Males and Females?

We would expect Dsx to be expressed in the cells where it is acting, i.e. the SGPs. However, the exact timing and role of Dsx in cell fate specification is unknown. We therefore used GFP-tagged Dsx, and visualized the SGPs via antibody staining with Traffic jam (Tj), to characterize the sex-specific expression of Dsx and its relation to SGPs.

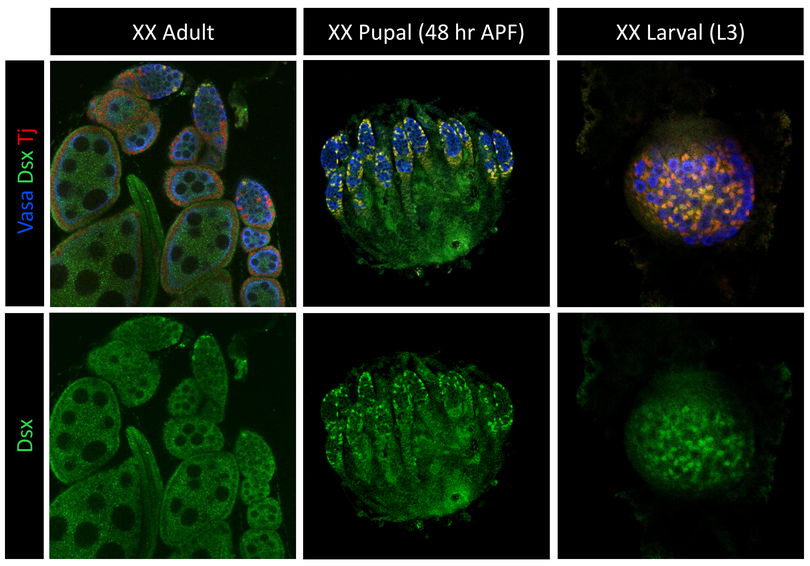
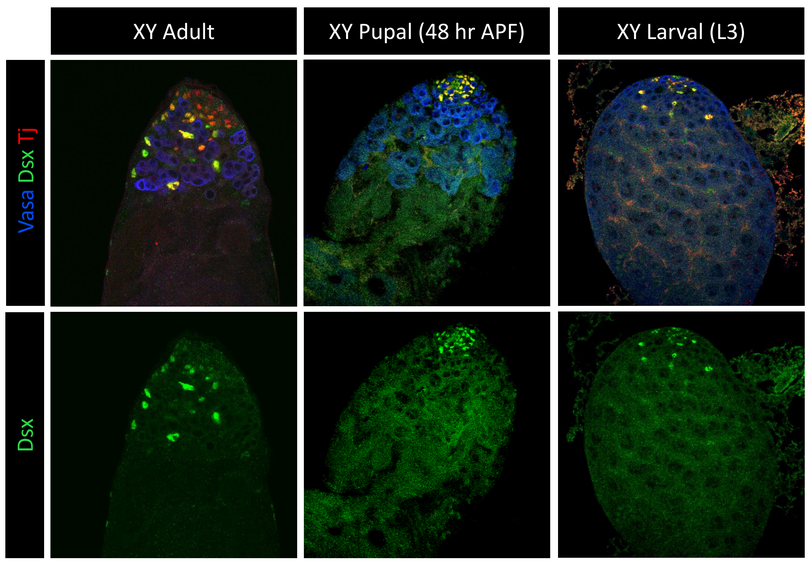


Fig 1. (above) The expression of Dsx in females shuts off as development progresses. In the L3 larval stage, Dsx is expressed in nearly every SGP. By 48 hours APF (after pupal formation), Dsx is seen in the cap cells, escort cells, and follicle cells. In the adult, Dsx expression is limited to the cap cells and escort cells.

Fig 2. (below) Dsx expression in males persists throughout development. In the L3 larval stage, Dsx is expressed in the early cyst cells. This Dsx expression is still seen at 48 hours APF and in the adult.

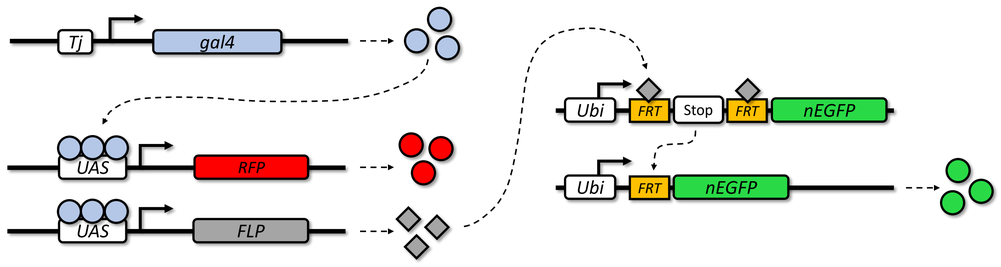


Where is Dsx Expressed in Males and Females?

- Follicle cells and cyst cells are analogous to one another, yet one appears to tolerate the loss of Dsx (females) while the other (males) does not
- Do follicle cells only need an early pulse of Dsx^F to specify their fate, and cyst cells need constant Dsx^M in order to remain male? Which cell types autonomously require *dsx* and *tra?*
- We will examine earlier developmental time points to look at when and where Dsx expression is first seen
- We will induce *dsx* LOF clones early in development and examine adult gonads to determine which cell types are still present and normally developed; similar analyses will be performed with sex-switched *dsx* isoforms

Which Cells Come From the SGPs?

We have previously characterized how SGPs contribute to testis development, but little is known regarding ovarian sexual development; how do the SGPs, and possibly other cells migrating into the gonad, contribute to the greater number of cell types in the developing ovary as compared to the testis? And are these the same cells where we see Dsx expression?



We used a technique called G-TRACE (diagramed above), which permanently labels SGPs and allows for analysis at desired time points. In brief, the SGP marker, Tj, is used to drive UAS-*RFP* for visualization of real-time expression and Ubi-*GFP* to show lineage expression. This was used in combination with Vasa antibody staining to visualize germ cells, as germ cells would be expected to never have any lineage or real-time expression.

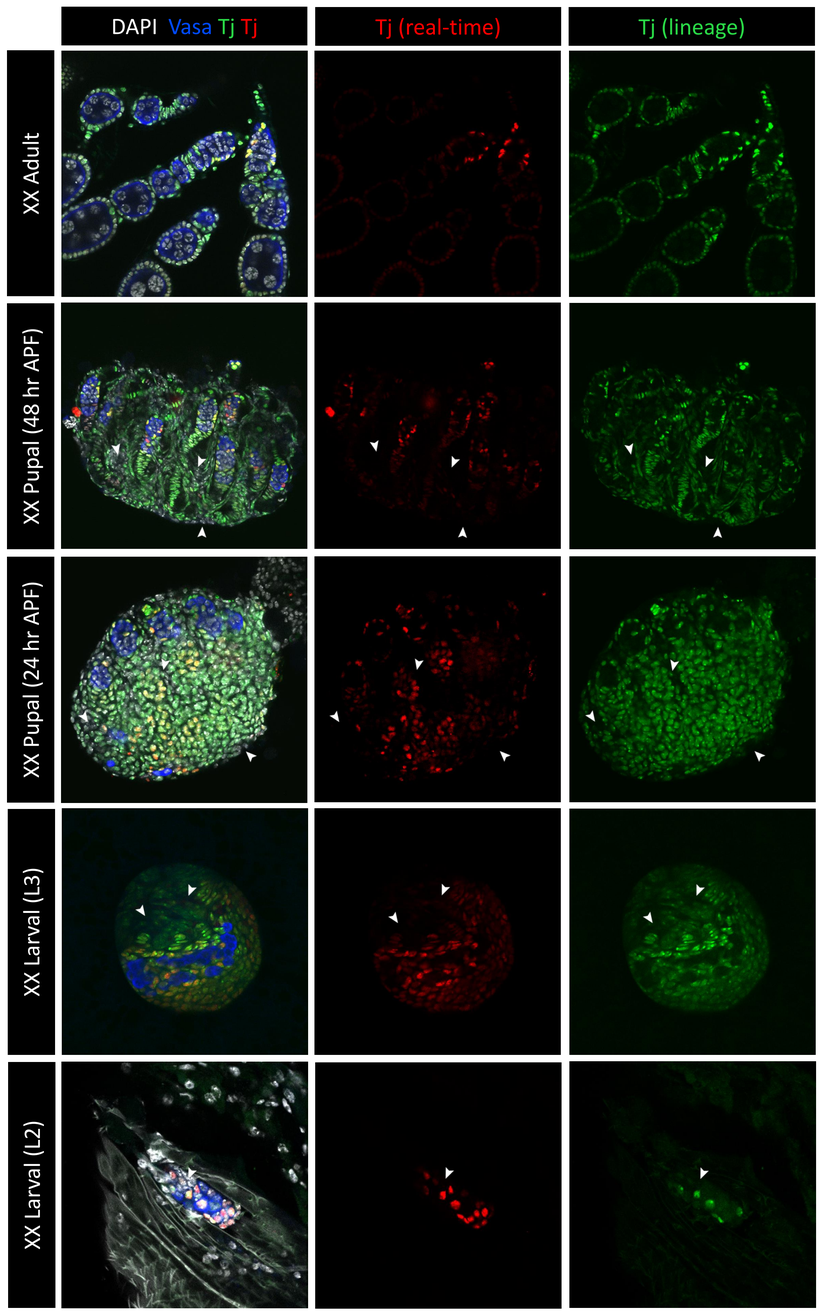


Fig 3. (above) We found cells with neither lineage nor real-time expression, which do not coincide with the germ cells (arrows). In the L3 larval stage, lineage expression is seen in the developing terminal filaments and nearly all of the intermingled cells (which go on to become escort cells and follicle cells), however some unlabeled cells are seen in the apical cap. By 48 hours APF, lineage expression is seen in the terminal filaments, cap cells, escort cells, follicle cells, and basal stalk cells; unlabeled cells are seen between germaria. In the adult, the unlabeled somatic cells are no longer visible in the germarium, and lineage expression is seen in the terminal filaments, cap cells, escort cells, follicle cells, and stalk cells.

Which Cells Come From the SGPs?

- The presence of unlabeled Vasa-negative cells indicates that there are non-SGP descendant somatic cells in the ovary
- Terminal filament cells are presumed to emerge from the apical cap at late larval stages, but the bright lineage expression observed here points to their specification even earlier in development
- Do non-SGPs migrate from other tissues to contribute to the somatic gonad? Does Tj turn on Dsx?
- We will investigate SGP lineage expression using G-TRACE at earlier developmental time points to create a temporal map of somatic cell specification and differentiation
- We will use G-TRACE with tissue-specific drivers to determine where the non-SGPs could be migrating from

Acknowledgements

I would like to thank my advisor, Mark, as well as all the other members of the Van Doren lab for their discussions and support. Special thanks to the Integrated Imaging Center (IIC) at JHU, the Johnston Lab, the Chen Lab, and the Trcek Lab.